DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR THE ASSAY OF NEVIRAPINE AND ITS RELATED SUBSTANCES IN TABLET DOSAGE FORM

Dissertation submitted to

THE TAMILNADU Dr. M. G. R. MEDICAL UNIVERSITY,

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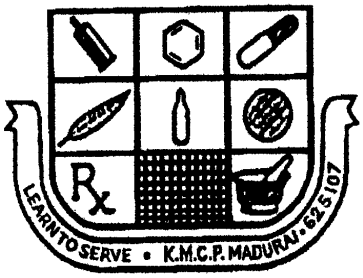
in partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS



DEPARTMENT OF PHARMACEUTICAL ANALYSIS

K. M. COLLEGE OF PHARMACY

UTHANGUDI, MADURAI – 625107

APRIL - 2012

CERTIFICATE

This is to certify that the dissertation entitled "**DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR THE ASSAY OF NEVIRAPINE AND ITS RELATED SUBSTANCES IN TABLET DOSAGE FORM**" submitted by **Mr. SOMAVEL. G** (**Reg. No: 26101728**) in partial fulfillment of the degree of Master of Pharmacy in Pharmaceutical Analysis, **K. M. COLLEGE OF PHARMACY**, Madurai – 625107 under **THE TAMILNADU DR. M. G. R. MEDICAL UNIVERSITY**, Chennai, carried out in EDICT PHARMACEUTICALS, Chennai.

It is a bonafide work carried out by him under my guidance and supervision during the academic year 2011 – 2012. This dissertation partially or fully has not been submitted for any other degree or diploma of this University or any other Universities.

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TO WHOMSOEVER IT MAY CONCERN

This is to certify that **Mr.SOMAVEL.G** of **KM COLLEGE OF PHARMACY, MADURAI** doing his **Final Year M.Pharmacy (Pharmaceutical Analysis)** has successfully completed his Training in **EDICT PHARMACEUTICALS PVT LTD.**

Project was focused on **NEWER ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF NEVIRAPINE IN TABLETS DOSAGE FORM** for a period of **June 2011 – November 2011**.

During this period his conduct is very good.

For **Edict Pharmaceuticals Pvt Ltd**

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Somavel.

G

CONTENTS

ABBREVIATIONS

ABS	–	Absorbance
ACN	–	Acetonitrile
API	–	Active Pharmaceutical Ingredient(s)
AR	–	Analytical reagent
C_{max}	–	Maximum concentration
Conc	–	Concentration
CRS	–	Control resolution sample
DSC	–	Differential scanning calorimeter
Edn	–	Edition
e.g.	–	Example gratitis
eq	–	Equation
FDA	–	Food and Drug Administration
Fig.No	–	Figure Number
GC	–	Gas Chromatography
GC-ICP-MS	–	Gas chromatography - inductively coupled plasma - mass spectroscopy
GC-IR	–	Gas chromatography - Infrared spectroscopy
GC-MS	–	Gas chromatography - Mass spectroscopy
gm	–	Gram
HIC	–	Hydrophobic interaction chromatography
HILIC	–	Hydrophilic interaction liquid chromatography
HPCCC	–	High performance counter current chromatography
HPTLC	–	High performance thin layer chromatography
HSCCC	–	High speed counter current chromatography

ICH	–	International Conference on Harmonization
ICP-MS	–	Inductively coupled plasma-Mass spectroscopy
Imp	–	Impurity
Inj	–	Injection
IS	–	Internal standard
ISBN	–	International standard book number
LC-MS	–	Liquid chromatography - Mass spectroscopy
LC-NMR	–	Liquid chromatography - Nuclear mass resonance spectroscopy
LOD	–	Limit of Detection
LOQ	–	Limit of Quantitation
M	–	Molar
Max	–	Maximum
mg	–	Milligram
min	–	Minute
mL	–	Millilitre
mM	–	Millimolar
NA	–	Not Applicable
nm	–	Nanometer
NMT	–	Not More Than
NPC	–	Normal phase chromatography
NVP	–	Nevirapine
ODS	–	Octa decyl silane
PDA	–	Photodiode array
PI	–	Protease inhibitors
Rel.Comp	–	Nevirapine Related Compound

RP-HPLC	–	Reverse phase high performance liquid chromatography
RS	–	Reference standard
RSD	–	Relative standard deviation
Rt	–	Retention time
S.No	–	Serial number
SD	–	Standard deviation
SFC	–	Supercritical fluid chromatography
STD	–	Standard
TGA	–	Thermo gravimetric analysis
TLC	–	Thin layer chromatography
UPLC	–	Ultra performance liquid chromatography
UV	–	Ultraviolet
t_{max}	–	Maximum time
^{o}C	–	Degree centigrade
%	–	Percentage
µ	–	Micron
µg	–	Microgram
µm	–	Micrometer
θp	–	Purity angle
θth	–	Purity threshold

1. INTRODUCTION

Pharmaceutical analytical chemistry is concerned with the identification of a substance, the elucidation of its structure and quantitative analysis of its composition. It is an interdisciplinary branch of science which deals with various disciplines of chemistry such as inorganic, organic, physical, industrial and biochemistry. It also finds extensive application in environmental science, agricultural science and clinical chemistry, solid state research and electronics, oceanography, forensic science and space research. The scope of analytical chemistry is very broad and embraces a wide range of manual, chemical and instrumental techniques. [1]

Pharmaceutical analyst in research and development (R&D) of pharma industry plays a very comprehensive role in new drug development and follow up activities to ensure that, a new drug product meets the established standards, its stability and continued to meet quality throughout its shelf life.

The different activity of R&D includes drug development, (synthesis and manufacture), formulation, clinical trials, evaluation and finally launching i.e., finished products. Closely associated with these processes are regulatory and quality assurance functions.

1.1 CLASSIFICATION OF ANALYTICAL TECHNIQUES

The discipline involves qualitative analysis and quantitative analysis

- **Qualitative Analysis:** deals with identification of the substance [2]
- **Quantitative analysis**: deals with determination of amount of constituent present.

The applied science of analytical chemistry is instrumental analysis, which involves the study of theoretical principle of various instrumental methods.

In this the physical property of a substance is measured to determine its chemical composition. These methods save the time and avoid chemical separation and give increased accurate results.

The instrumental methods can be categorized into five techniques are tabulated in table no. 1 as follows [3]

Table No: 1 Classification of instrumental analytical methods

Spectro photometric Techniques	Electro-Chemical Techniques	Chromato graphic Techniques	Miscellaneous Techniques	Hyphenated Techniques
Colorimetry	Conductometry	HPLC	Thermal analysis (TGA & DSC)	LC-MS
UV-Visible spectroscopy	Potentiometry	UPLC		GC-MS
Fluorescence and phosphorescence spectroscopy	Coulometry	GC		GC-IR
Atomic spectroscopy	Voltammetry	TLC		LC-MS/MS
IR spectroscopy	Electro-gravimetry	HPTLC		LC-NMR
X- ray radiation		SFC		ICP-MS
Mass spectroscopy		TLC		GC-ICP-MS
NMR spectroscopy				LC-NMR
Turbidimetry and Nephelometry				
Electron spin resonance spectroscopy				

1.2 CHROMATOGRAPHY

Chromatography is a technique in which solutes are resolved by differential rates of elution as they pass through a chromatographic column. Chromatography is essentially a group of techniques for the separation of the compounds of mixtures by their continuous distribution between two phases, one of which moves relative to the other. [4]

History:

Many publications have discussed or detailed the history and development of chromatography. Table No.2 lists the chronological order of the events that are the most notable in the development of the present state of the field. Since the various types of chromatography (liquid, gas, paper, thin-layer, ion exchange, supercritical fluid, and electrophoresis) have many features in common, they must all be considered in development of the field. [5]

Table No: 2 Development of the chromatography

Year	Scientist	Research findings
1834	Runge .F.F	Used unglazed paper and / or pieces of cloth for spot testing dye mixtures and plant extracts
1850	Runge .F.F	Separated salt solutions on paper
1868	Goppelsroeder,F.	Introduced paper strip (capillary analysis) analysis of dyes, hydrocarbons, milk, beer, colloids, drinking and mineral water, plant and animal pigments
1906-1907	Twsett,M.	Separated chloroplast pigment on $CaCO_3$ solid phase and petroleum ether liquid phase
1931	Kuhn,R.et.al	Introduced liquid-solid chromatography for separating egg yolk xanthophylls

1940	Wilson,J.N	Wrote first theoretical paper on chromatography: assumed complete equilibration and linear sorption isotherms, qualitatively defined diffusion, rate of adsorption, and isotherm non linearity
1941	Tiselius,A.	Developed liquid chromatography and pointed out frontal analysis, elution analysis, and displacement development
1944	Consden, R.,Gordon,A.H., & Martin, A.J.P	Developed paper chromatography
1946	Claesson,S.	Developed liquid-solid chromatography with frontal and displacement development analysis
1951	Cremer,E.	Introduced gas-solid chromatography
1952	James,A.T.,and Martin,A.J.P.	Introduced gas-liquid chromatography
1957	Golay,M	Reported the development of open tubular columns

High Performance Liquid Chromatography

High performance liquid chromatography is the fastest growing analytical technique for the analysis of the drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for an analysis of many drugs in both dosage forms and biological fluids. HPLC was developed in the late 1960s and 1970s. Today it is widely accepted separation technique for both sample analysis and purification in variety of areas. The successful use of liquid chromatography requires the right combination of a variety of operating conditions such as the type of,

- Column packing.
- Mobile phase and its flow rate.
- Column length and diameter.
- Column temperature and sample size.

General uses of HPLC

1. Separation of wide variety of compounds, organic, inorganic and biological compounds, polymers, chiral compounds, thermally liable compounds and small ions to macro molecules.
2. Analysis of impurities.
3. Analysis of both volatile and nonvolatile compounds.
4. Determination of neutral ionic or zwitter ionic molecules.
5. Isolation and purification of compounds.
6. Ultra trace to preparative and process scale separations.
7. Qualitative and quantitative method.[6]

Guide to Liquid Chromatography Mode Selection

Selection of chromatography mode is based upon the analyte polarity, solubility and ionic nature. [7] A guide to liquid chromatography mode selection is represented in fig no: 1

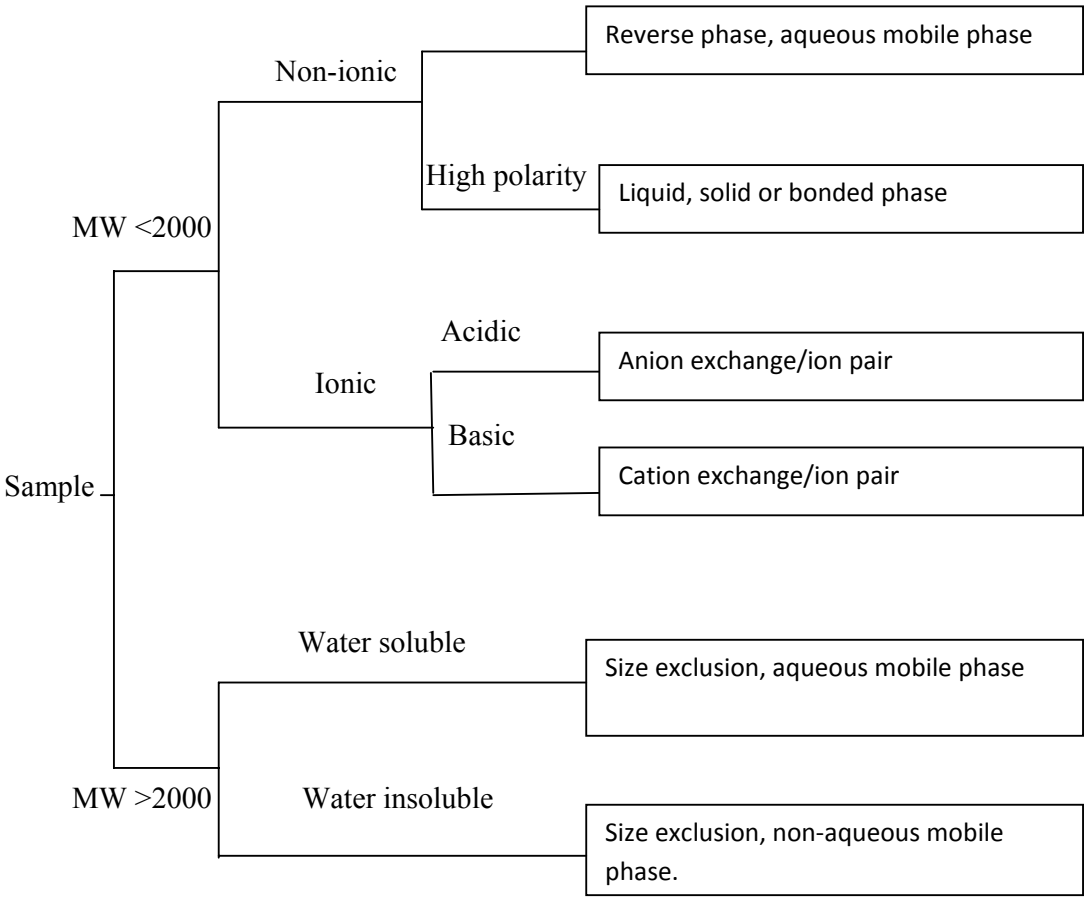


Fig. No: 1 Guide to Liquid Chromatography Mode Selection

Reverse Phase HPLC

Reverse phase chromatography refers to the use of a polar mobile phase with the non-polar stationary phase in contrast to normal phase chromatography where a polar stationary phase is employed with a non-polar mobile phase. [8] The figure No. 2 and 3 shows the HPLC system and its various components.

Fig.No: 2 High Performance Liquid Chromatography

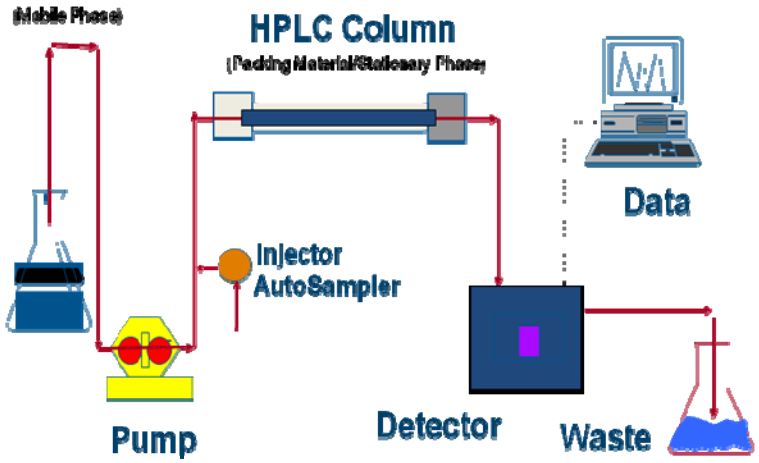
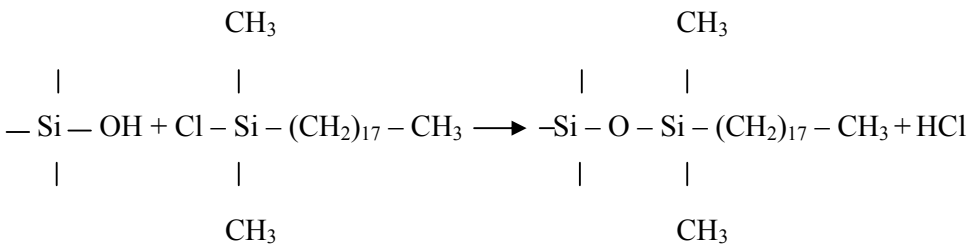


Fig.No: 3 Block diagram showing the components of an HPLC instrument

Reverse phase column packing

The liquid-liquid partition chromatography uses a stationary phase consisting of liquid layer adsorbed to a surface of silica or alumina. In case of bonded phase or reverse phase HPLC uses a stationary phase consisting of an organic moiety chemically bonded to the surface of silica through the surface silanol groups. Since the organic moieties are generally long-chain hydrocarbons, the mobile phases are generally polar. In this mode, the more polar solutes are eluted first while the non-polar compounds are eluted later. The silanol group can react with a chlorosilane group to form the popular (ODS & C_{18}) octadecylsilane packing.

E.g. Octadecylsilane (ODS or C_{18}).



For silica based bonded materials, the suitable working pH range is 2 - 8. At pH values of less than 2, the Si-C bond is attached, and at the higher pH values, hydrolysis of the siloxane takes place which leads to degradation or destruction of the packing. In most of the applications of RP-HPLC, elution is carried out with highly polar solvents such as methanol, acetonitrile or tetrahydrofuran in various concentrations. [9a]

Reverse phase mobile phase

The primary constituent of RP-HPLC mobile phase is water. Water miscible solvents such as methanol, ethanol, acetonitrile, dioxane, tetrahydrofuran and dimethyl formamide are added to adjust the polarity of the mobile phase. Additionally acids, bases, buffers and/or ionic surfactants are added. The water should be of high quality, either distilled or demineralised water.

The most widely used organic modifiers are methanol, acetonitrile and tetrahydrofuran. Methanol and acetonitrile have comparable polarities but acetonitrile is an aprotic solvent. Ethanol, 1-propanol and 2-propanol are also useful but less polar than methanol. Dioxane, tetrahydrofuran are aprotic solvents that are less polar than

acetonitrile. Reverse phase mobile phases are generally non-flammable due to high water content. Degassing is quite important with reverse phase mobile phases. [9b]

Detectors in HPLC

Characteristics of ideal detectors

1. Adequate sensitivity.
2. Good stability and reproducibility.
3. A linear response to analyte.
4. A short response time that is independent of flow rate.
5. High reliability.
6. Minimal internal volume in order to zone broadening. [9c]

Liquid chromatographic detectors are of two basic types,

1. Differential or bulk property detector
2. Selective or solute property detector

Differential or bulk property detector

Differential or bulk property detector provides a differential measurement of a bulk property that is possessed by both the analyte and the mobile phase.
e.g., Refractive index, dielectric constant.

Selective or solute property detector

Measure the property of an analyte which is not possessed by the mobile phase.
e.g., UV absorbance, fluorescence.

1.3 RELATED SUBSTANCES AND IMPURITIES

Preface

The pharmaceutical industry is required by the Food, Drug and Cosmetic Act to establish the identity and purity of all marketed drug products. The United States Food and Drug Administration (FDA) and other regulatory bodies around the world insist on the requirement of impurities in drug substance and drug product when present at threshold level recommended by the International Conference on Harmonisation (ICH) should be isolated and characterized. [10]

The identification of process related impurities and degradation products can provide an understanding on production of impurities and define degradation mechanism.

Definitions

Impurity: "any entity of the drug substances (bulk material) or drug (final container product) that is not the chemical entity defined as the drug substance, an excipient, or other additives to the drug product".

Degradation product: The compound produced as a result of decomposition of the material of interest or Active Pharmaceutical Ingredient are often called degradation products (degradants). Degradation could occur as a result of changes during processing or storage (e.g., oxidation, aggregation and proteolysis).

In the pharmaceutical world, an impurity is generally considered as any other organic material besides the drug substance or Active Pharmaceutical Ingredient, that arises out of synthesis. Most of the time, the inorganic contaminants are not given adequate consideration as impurities unless they are toxic, such as heavy metals or arsenic.

Organic Volatile Impurities (OVI) which are usually residual solvents as well as other organic volatile impurities used in the synthesis are often considered virtual impurities.

Interaction products are produced during formulation processes and degradation products.

Therefore, for all intents and purposes, various contaminants mentioned here can be called impurities and should be labelled as such because they decrease the purity of API.

Isolation and characterization of impurities

Impurities are generally assumed to be inferior to API because they might not have the same level of pharmacological activity. However, they are not necessarily always inferior. From the standpoint of its usage, the drug substance is not compromised in terms of purity even if it contains another material with superior pharmacological or toxicological properties. Therefore, any extraneous material present in the drug substances or active ingredients must be considered as an impurity even if it is totally inert or has superior pharmacologic properties so that an appropriate evaluation of its content in the drug product can be made.

In many final pharmaceutical products, test for related substances is performed as a part of the chemical quality control. This test is intended to check that the content of impurities structurally related to the active pharmaceutical ingredients (related substances), are at a very low level. This is important, as related substances can affect the efficacy of the final pharmaceutical product especially, degradation products from the API.

Identification and determination

The first step of impurity profiling is the detection of the impurities. High resolution NMR and mass spectrometry can play an important role in getting a finger print on the purity of the sample.

Impurity profiling in synthetic drug research

The use of analytical method is of utmost importance in all phases of synthetic research and other related areas (biotechnology, extraction of materials of plant and animal origin) aimed to introduce new chemical entities in therapy.

During the course of drug development, the qualititative impurity profile may change as a result of, for example, changes in synthetic route, drug product formulation, storage conditions and container / closure system. These changes result in increased levels of previously observed impurities above the identification and/or qualification threshold limits or new impurities not previously identified or qualified. When these impurities are above the threshold limits, consideration for identification and qualification of the level of the impurity is indicated and one of the decision trees for safety studies should be consulted. Safety studies should compare the new drug substance containing new a representative level of the impurity with previously qualified materials. Although studies using the isolated impurity are also acceptable, validated method for the separation and quantification of different impurities are preferred.

Various terms of impurities

- By product
- Degradation product
- Interaction product
- Intermediate
- Related product
- Transformation product
- Toxic impurities
- Organic volatile impurities
- Foreign substances
- Enantiomeric impurities

ICH Terminology

According to ICH guidelines, impurities are classified into the following three categories for the drug substance produced by chemical synthesis.

1. Organic impurities (starting materials, process related products, intermediates and degradation products).
2. Inorganic impurities (salts, catalyst, ligands and heavy metals or other residual metals).

3. Residual solvents (organic and inorganic liquids used during production and/or recrystallization).

Table No: 3 ICH Quality Guidelines

▪ **Q1 A**	- Stability testing of new drug substances and products
▪ **Q2 A**	- Validation of analytical procedures
▪ **Q3 A**	- Impurities in drug substances
▪ **Q3 B**	- Impurities in new drug products
▪ **Q6 A**	- Specifications: Test procedures and acceptance criteria for new drug substances and new drug products

Any impurity that may be present in the starting material has the potential to be carried into the active ingredient of interest.

The most obvious source of impurities is the synthesis, where intermediates and by-products may be carried into the API as impurities or an intermediate and become a source of other impurities resulting from them.

1.4 VALIDATION

The word "Validation" means "Assessment" of validity or "action of proving effectiveness". [11]

System Suitability

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

The system suitability testing parameters established for the liquid chromatographic procedure are:

- Retention time
- Symmetry factor or tailing factor
- Theoretical plates
- Resolution

General considerations

Retention Time (t)

This is the time of emergence of the peak maximum of a component after injection.

Symmetry factor (or) Tailing factor (T)

$$T = W / 2f$$

The assessment of peak shape is in terms of symmetry factor.

W - Width of peak at 5 % height

f - Distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5 % of the peak height from the baseline (or) width of front half of the peak at 5 % height

Number of theoretical plates (N)

$$N = 5.54\ T / W$$

The assessment of performance of efficiency of a column is in terms of the number of theoretical plates.

W - Width of peak at half height

Resolution (R)

$$R = 2\ (t_2 - t_1) / W_2 + W_1$$

This gives the resolution between the measured peaks on the chromatogram

Where,

t_2 and t_1 - Retention times of the two components.

W_2 and W_1 - Widths of the components at the bases of the peaks obtained by extrapolating the relatively straight sides of the peaks to the baseline.[12]

2. DRUG PROFILE

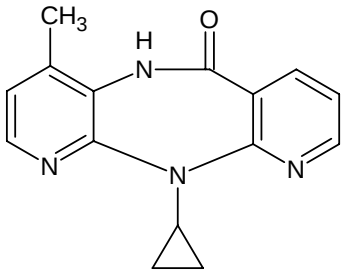
Name

Nevirapine

IUPAC Name

11-Cyclopropy1-5, 11-dihydro-4-methy1-6H-dipyrido [3, 2-b : 20, 30-e] [1, 4] diazepin-6-one. [13]

Structure of Nevirapine



Molecular Formula : $C_{15}H_{14}N_4O$

Molecular Weight : 266.30

CAS Number : 129618-40-2

Category : Non-nucleoside reverse transcriptase inhibitor (NNRTI), class of anti-retroviral.

Description : White or almost white, odorless to nearly odorless, crystalline powder.

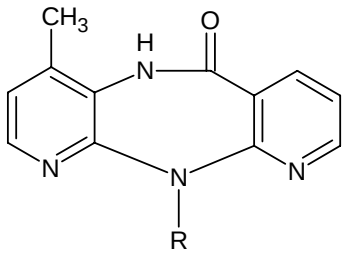
Solubility : Practically insoluble in water, slightly soluble in alcohol and in methanol.

Storage : Store at 25 °C (77 °F), excursions permitted to 15 °C-30 °C (59 °F-86 °F).

History:

Nevirapine was discovered by Hargrave et al. at Boehringer Ingelheim Pharmaceuticals, Inc., one of the Boehringer Ingelheim group of companies. It is covered by U.S. Patent 5,366,972 and corresponding foreign patents. Nevirapine was the first NNRTI approved by the U.S. Food and Drug Administration (FDA). It was approved June 21, 1996 for adults and september 11, 1998 for children. [14] At present nevirapine is an official drug in Indian Pharmacopoeia [15], British Pharmacopoeia [16] and United States Pharmacopoeia.[17]

Impurities **:** Specified impurities A, B and C. [16]



Related Substance A. **R** = C_2H_5 : 11-ethyl-4-methyl-5, 11-dihydro-6H-dipyrido [3, 2-b: 2',3'-e] [1,4] diazepin-6-one.

Related Substance B. **R** = H : 4-methyl-5, 11-dihydro-6H-dipyrido [3, 2- b: 2',3'-e] [1, 4] diazepin-6-one.

Impurity C. **R** = CH_2-CH_2-CH_3 : 4-methyl-11-propyl-5, 11-dihydro-6H-dipyrido [3, 2-b : 2', 3'-e] [1,4] diazepin-6-one.

Dosage and administration: 200 mg tablets - Oral route of administration

Half life **:** 25 - 30 hours

Brand Names : Nevimune 200 mg, Nevivir 200 mg

Mode of action

Nevirapine diffuses into the cell and binds to reverse transcriptase adjacent to the catalytic site. This induces conformational changes that inactivate the enzyme. Resistance develops rapidly in cells exposed to nevirapine. High-level resistance is associated with mutations at reverse transcriptase codons 101, 103, 106, 108, 135, 181, 188 and 190.

Pharmacokinetics:

Absorption

Nevirapine is well absorbed orally. It is readily crosses the placenta and has been found in breast milk, a feature that has encouraged use of nevirapine for pprevention of mother-to-child transmission of HIV. 90 % (Absolute bioavailability 93 ± 9 %), CSF to plasma level is 45 %. [18]

Distribution

Nevirapine is highly lipophilic and is essentially non-ionized at physiological pH.

Metabolism

Oxidative metabolism of nevirapine in the liver by cytochrome P_{450} isoforms CYP3A4 and CYP2B6 produces several metabolites including 2 -, 3 -, 8 - and 12 - hydroxynevirapine.

Elimination

Cytochrome P_{450} metabolism, glucuronide conjugation and urinary excretion of glucuronidated metabolites represent the primary route of nevirapine biotransformation and elimination.

Drug Interactions:

Rifamibicin and ketoconazole are contraindicated in patients receiving nevirapine. Plasma ethinyl estradiol level decreases significantly with nevirapine coadministration. Although it can lower plasma concentrations of protease inhibitors. [19]

3. LITERATURE REVIEW

1. **Anand Babu K, *et al.*, (2011)** [20] have developed and validated method for the simultaneous estimation of zidovudine, lamivudine and nevirapine tablets by RP-HPLC.

Column : Hypersil BDS C_{18} column
Mobile Phase : Ammonium acetate buffer : acetonitrile [75 : 25]
Flow Rate : 1 mL/min
Detector : UV detector set at 270 nm

2. **D'Avolio A, *et al.*, (2011)** [21] have validated a sensitive and accurate high performance liquid chromatography-mass spectrometric (HPLC-MS) method for the intracellular determination of 14 anti-retroviral drugs in peripheral blood mononuclear cells (PBMCs) for HIV patients.

Column : Atlantis T_3 column
Mobile Phase : Gradient elution water (0.05% formic acid) and ACN
Flow Rate : 1 mL/min
Detector : UV detection at 284 nm

3. **Rohini, *et al.*, (2011)** [22] have estimated a reverse phase high performance liquid chromatography method for nevirapine in tablets.

Column : Symmetry C_{18} column
Mobile Phase : Acetonitrile : phosphate buffer [65 : 35]
Flow Rate : 0.8 mL/min
Detector : UV detection at 283 nm

4. **Shewiyo D H, *et al.*, (2011)** [23] have developed and validated an improved method for the simultaneous analysis of lamivudine, stavudine and nevirapine using high performance thin layer chromatography (HPTLC).

Column : $C_{18\ C}$olumn
Mobile Phase : Ethyl acetate : methanol : toluene : ammonia [38.7 : 19.4 : 38.7 : 3.2]
Detector : Densitometric detection

5. **D'Avolio A, *et al.*, (2010)** [24] have developed a bioanalytical method for the determination of most commonly prescribed protease inhibitors (saquinavir, atazanavir, amprenavir, darunavir, lopinavir and ritonavir) and non-nucleoside reverse transcriptase inhibitors (etravirine, efavirenz and nevirapine). C_{18} column was used to analyse the samples.

6. **Else L, *et al.*, (2010)** [25] described a simple, fast and sensitive HPLC-MS/MS method for determination of the commonly used protease inhibitors (PI) amprenavir, atazanavir, darunavir, lopinavir, ritonavir, saquinavir and the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine.

   Column **:** C_{18} column

   Mobile Phase **:** Gradient acetonitrile and 0.05% formic acid

Detector **:** UV detection at 280 nm

7. **Hemanth Kumar A K, *et al.*, (2010)** [26] have described a relatively simple and accurate method to estimate anti-retroviral drugs in pharmaceutical preparation is by spectrophotometric method.

   Column **:** C_{18} column

   Detector **:** UV detection at 300, 285 and 270 nm

8. **Kaale E, *et al.*, (2010)** [27] have developed an inter-laboratory investigation on the use of high performance thin layer chromatography to perform assays of lamivudine, zidovudine, metronidazole, nevirapine, and quinine composite samples.

9. **Li Z, *et al.*, (2010)** [28] have developed a new high throughput LC-MS/MS method for the simultaneous determination of lamivudine, stavudine and nevirapine in human plasma with zidovudine as an internal standard. Shiseido C_8 column was used.

10. **Ren C, *et al.*, (2010)** [29] have developed a highly sensitive and specific LC-MS/MS assay method and validated to quantify nevirapine and its five metabolites [2-, 3-, 8-, 12-hydroxyl NVP and 4-carboxyl NVP] simultaneously in baboon serum.

11. **Vogel M, *et al.*, (2010)** [30] have described a simple, stable and specific gas chromatography-mass spectrometry method for the determination of nevirapine in plasma.

12. **Elens L, *et al.*, (2009)** [31] have reported an ultra performance liquid chromatography (UPLC) diode array detection method for the simultaneous quantification of the HIV protease inhibitors amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, tipranavir and the non-nucleoside reverse transcriptase inhibitors efavirenz and nevirapine. C_8 column was used to analyse the sample.

13. **Malm M, *et al.*, (2009)** [32] have developed and validated a bioanalytical method for the determination of lamivudine, zidovudine and nevirapine in 100 µL capillary blood applied onto sampling paper.

    Column **:** Zorbax SB C_8 column

    Mobile Phase **:** 2 step gradient: methanol : sodium acetate buffer [pH 3.95, 15 : 85] and [pH 3.95, 50 : 50]

    Flow Rate **:** 1.0 mL/min

    Detector **:** UV detection at 265 nm

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14. **Martin J, *et al.*, (2009)** [33] have described a liquid chromatography-tandem mass spectrometry assay for simultaneous determination of the plasma concentration of 11 anti-retroviral agents (nevirapine, indinavir, atazanavir, amprenavir, saquinavir, ritonavir, lopinavir, efavirenz, tipranavir, darunavir and maraviroc).

15. **Mandloi D K, *et al.*, (2009)** [34] have developed a simple, highly sensitive, isocratic reverse phase high performance liquid chromatography method for the determination of nevirapine in the bulk drug and tablet dosage forms.

Column **:** Luna 5 µ, C_{18} column

Mobile Phase **:** Methanol and water [89 : 11]

Flow Rate **:** 1 mL/min

Detector **:** UV detection at 284 nm

16. **Nageswara Rao R, *et al.*, (2009)** [35] have developed and reported a simple, rapid, reliable and highly sensitive online two-dimensional reverse phase liquid chromatography-tandem mass spectrometric method to determine anti-retroviral drugs viz., abacavir, nevirapine and indinavir in rat serum and urine.

Column **:** C_{18} column

Detector **:** ESI-MS/MS detection

17. **Prasada Rao C H, *et al.*, (2009)** [36] have developed a reverse phase high performance liquid chromatography method for the estimation of nevirapine in bulk drug and pharmaceutical dosage forms.

Column **:** Octa decyl silane column

Mobile Phase **:** Methanol and acetate buffer [60 : 40]

Flow Rate **:** 1.0 mL/min

Detector **:** UV detection at 280 nm

18. **Purnima D. Hamrapurkar, *et al.*, (2009)** [37] have reported a validated method for the estimation of nevirapine from bulk drug and tablet formulations using appropriate solvent system on TLC plates.

HPTLC plates **:** Precoated silica gel, GF254, 10 cm x 20 cm

Solvent **:** Ethyl acetate : glacial acetic acid (10 : 0.1)

Detection **:** Densitometrically scanned at 283 nm

19. **Stieger N, *et al.*, (2009)** [38] have developed UV spectrophotometric method for the quantitative determination of nevirapine in water, methanol and 0.1 N HCl.

20. **D'Avolio A, *et al.*, (2008)** [39] have developed and validated a new method using high-performance liquid chromatography coupled with PDA detector for the quantification of plasma concentrations of 11 anti-retroviral agents including nevirapine and its metabolites.

21. **Heine R, *et al.*, (2008)** [40] have developed a bioanalytical method for the determination of most commonly prescribed protease inhibitors (atazanavir,

darunavir, lopinavir and ritonavir) and non-nucleoside reverse transcriptase inhibitors (efavirenz and nevirapine).

Column **:** C_{18} column (150 mm x 2.0 mm)

Mobile Phase **:** Acetonitrile, methanol and $ZnSO_4$ in water [1 : 1 : 2]

22. **Notari S, *et al.*, (2008)** [41] have quantified abacavir, amprenavir, didanosine, efavirenz, nevirapine and stavudine in the plasma of HIV infected patients, by standard addition analysis.

23. **Palaniappan Mohanraj, *et al.*, (2008)** [42] have described the method for determination of nevirapine in tablet dosage forms by simple and rapid RP-HPLC method.

    Column **:** ODS column

    Mobile Phase **:** Methanol and water [89 : 11]

    Flow Rate **:** 1 mL/min

    Detector **:** UV detection at 284 nm

24. **Gehrig A K, *et al.*, (2007)** [43] have developed a bioanalytical method for determination of some of the anti-HIV drugs including nevirapine.

    Column **:** Nucleosil C_{18} column

    Mobile Phase **:** Gradient 20 mM ammonium acetate including 0.1 % aqueous acetic acid and acetonitrile

    Detector **:** Electrospray ionisation / tandem mass spectrometry

25. **Herve Rebiere, *et al.*, (2007)** [44] have developed and validated two methods by reverse phase liquid chromatography for the analysis of 19 anti-retroviral molecules.

Method I: (NRTI)

Column **:** YMC pack ODS

Mobile Phase **:** Gradient ammonium acetate buffer pH 4.0 : methanol

Flow Rate **:** 1 mL/min

Detector **:** UV detection at 270 nm

Method II: (NNRTI & PI)

Column **:** Symmetry C_{18}

Mobile Phase **:** Gradient potassium phosphate buffer pH 5.6 : ACN

Flow Rate **:** 1.5 mL/min

Detector **:** UV detection at 260 nm

26. **Liu Z, *et al.*, (2007)** [45] have reported a rapid highly sensitive and specific electrospray ionization liquid chromatography/tandem mass spectrometry method for quantification of nevirapine and its two metabolites, 2-hydroxynevirapine and nevirapine 4-carboxylic acid.

27. **Mistri H N, *et al.*, (2007)** [46] have developed and validated a selective and high throughput liquid chromatography-tandem mass spectrometry method to detect and simultaneously quantify lamivudine, stavudine and nevirapine in human plasma using metaxalone as internal standard.

    Column **:** Symmetry C_{18}

    Mobile Phase **:** Water : acetonitrile [20 : 80]

28. **Rowland L S, *et al.*, (2007)** [47] have developed and validated a multiple reaction monitoring LC/MS/MS method for the analysis of nevirapine oxidative metabolites, 2-hydroxynevirapine, 2-hydroxynevirapine, 3-hydroxy nevirapine, 8-hydroxynevirapine, 12-hydroxynevirapine and 4-carboxy nevirapine in human plasma.

29. **Weller D R, *et al.*, (2007)** [48] have described an efficient, isocratic high performance liquid chromatography method for determining human immunodeficiency virus non-nucleoside reverse transcriptase inhibitors and protease inhibitors in plasma is advantageous for laboratories participating in clinical trials and therapeutic drug monitoring programs, or conducting small animal research.

    Column **:** S_3 column

    Detector **:** Detection at 212 nm

30. **Geetha Ramachandran, *et al.*, (2006)** [49] have described a reverse phase high performance liquid chromatographic method for simultaneous determination of plasma zidovudine and nevirapine.

Column **:** C_{18} column

Mobile Phase **:** Potassium dihydrogen phosphate pH 7.5 : acetonitrile [75 : 25]

Flow Rate **:** 1.5 mL/min

Detector **:** UV detection at 260 nm

31. **Notari S, *et al.*, (2006)** [50] have developed a HPLC-UV method to quantify simultaneously some HIV protease inhibitors, reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors including nevirapine in human plasma.

    Column **:** C_{18} Symmetry

    Mobile Phase **:** Linear gradient 0.01 M potassium dihydrogen phosphate and acetonitrile

    Flow Rate **:** 1.0 mL/min

    Detector **:** UV detection at 240 and 260 nm

32. **Namita Kapoor, *et al.*, (2006)** [51] have developed a method for the simultaneous determination of lamivudine, stavudine and nevirapine in anti-retroviral fixed dose combinations by high performance liquid chromatography.

    Column **:** Symmetry C_{18} column

    Mobile Phase **:** Gradient elution

    Sol. A: Acetate buffer pH 3.5: methanol [80 : 20]

    Sol. B: Acetonitrile : isopropyl alcohol [50 : 50]

Flow Rate **:** 0.6 mL/min

Detector **:** UV detection at 270 nm

33. **Ramachandran G, *et al.*, (2006)** [52] have described a simple, fast, isocratic, reverse phase high performance liquid chromatographic method for simultaneous determination of plasma zidovudine and nevirapine.

    Column **:** C_{18} column

    Mobile Phase **:** Potassium dihydrogen phosphate pH 7.5 and acetonitrile [72 : 28].

    Detector **:** UV detection at 260 nm

34. **Rezk N L, *et al.*, (2006)** [53] have reported full validation of an analytical method that combines atazanavir with HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors including nevirapine.

    Column **:** Zorbax C_{18}

    Detector **:** Detection at 210 nm

35. **Sarkar M, *et al.*, (2006)** [54] have developed and validated HPLC and an UV spectrophotometric method for the quantitative determination of three antiretroviral drugs viz., lamivudine, stavudine and nevirapine that constitute one of the first line regimens in antiretroviral therapy.

    Column **:** C_{18} symmetry

    Detector **:** UV detection at 270, 265 and 313 nm

36. **Silverthorn C F, *et al.*, (2006)** [55] have validated clinically relevant assay for the assessment of nevirapine concentrations in neonate plasma samples.

    Column **:** C_8 column

    Detector **:** UV detection at 280 nm

37. **Anbazhagan S, *et al.*, (2005)** [56] have described the simultaneous quantification of stavudine, lamivudine and nevirapine in tablets by UV spectroscopy, reverse phase HPLC and HPTLC methods.

    Column **:** C_{18} ODS-Hypersil column

    Mobile Phase **:** Sodium phosphate buffer : acetonitrile [4 : 1]

    Detector **:** UV detection at 266, 271 and 315 nm

38. **Colombo S, *et al.*, (2005)** [57] have described a sensitive and accurate liquid chromatography-tandem mass spectrometric method for the intracellular determination of nine antiretroviral drugs in human peripheral blood mononuclear cells (PBMCs). PBMCs are isolated by density gradient centrifugation using vacutainer CPT tubes and cell count is performed with a coulter instrument.

    Column **:** RP C_{18} symmetry shield

    Mobile Phase **:** Gradient elution ammonium acetate and acetonitrile

    Detector **:** UV detection

39. **Koal T, *et al.*, (2005)** [58] were quantified for the anti-retroviral drugs, i.e., PI and NNRTI in dried blood spots from HIV/AIDS patient whole blood samples as the basis for therapeutic drug monitoring by a robust simultaneous liquid chromatography/tandem mass spectrometry method. This study includes PI and NNRTI using phenomenex synergy max column.

40. **Lemmer P, *et al.*, (2005)** [59] have developed an accurate, selective, and sensitive method for the determination of the non-nucleoside reverse transcriptase inhibitors nevirapine and efavirenz in human plasma using gas chromatography-mass spectroscopy.

41. **Schuman M, *et al.*, (2005)** [60] have developed and validated a RP-HPLC method using photo diode array detection for the simultaneous quantification of lamivudine, stavudine, nevirapine, zidovudine, methylparaben and propylparaben in solid and liquid drug formulations.

    Column **:** Symmetry C_8 column

    Mobile Phase **:** Gradient 50 mM NaH_2PO_4 pH 3.8 and ACN

    Flow Rate **:** Gradient (0.5 to 1.0 mL/min)

    Detector **:** UV detection

42. **Tribut O, *et al.*, (2005)** [61] have reported simultaneous quantification of anti-retroviral drugs and it metabolites in human plasma.

    Column **:** X-TERRA column

    Mobile Phase **:** Gradient water (3 mM pyrrolidine) and acetonitrile

    Detector **:** UV detection

43. **Dailly E, *et al.*, (2004)** [62] have proposed a global method for therapeutic drug monitoring of atazanavir, a novel protease inhibitor along with all other protease inhibitors and non-nucleoside reverse transcriptase inhibitors which are currently used to treat HIV patients. All drugs are extracted after a liquid-liquid extraction and separated on a C_{18} column with a binary gradient elution

except lopinavir. The absorbance is measured at 259 nm except for lopinavir (205 nm) and nevirapine (320 nm).

44. **Dubuisson J G, *et al.*, (2004)** [63] described a fast, inexpensive thin-layer chromatography method to detect the presence of NVP in human plasma. Clinical samples with nevirapine concentrations predetermined by high performance liquid chromatography were used to validate the TLC method.

45. **Egge Jacobsen W, *et al.*, (2004)** [64] have reported an analytical assay based on automated sample preparation and liquid chromatography coupled with electrospray mass spectrometry for the quantification of some of the anti-viral drugs.

46. **Kaul N, *et al.*, (2004)** [65] have developed and validated sensitive, selective, precise and stability indicating HPTLC method of analysis of nevirapine both as a bulk drug and in formulations.

    Mobile Phase **:** Toluene : carbon tetrachloride: methanol: acetone : ammonia [3.5 : 3.5 : 2.0 : 1.0 : 0.05]

Detection **:** Densitometric analysis, absorbance mode at 289 nm

47. **Rezk N L, *et al.*, (2004)** [66] have described an accurate, sensitive, and specific reverse phase high performance liquid chromatography assay for the simultaneous quantitative determination of HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors in human blood plasma.

    Mobile Phase **:** Linear gradient phosphate buffer (pH 4.5), acetonitrile, methanol and TFA.

    Flow Rate **:** Gradient mobile phase flow rate (0.9 - 1.1 mL)

    Detector **:** UV detection

48. **Bregt S Kappelhoff, *et al.*, (2003)** [67] have developed method for the simultaneous determination of the NNRTI efavirenz and nevirapine in human plasma using liquid chromatography.

Column **:** Zorbax C_{18} column

Mobile Phase **:** Ammonium acetate : methanol

Flow Rate **:** 1 mL/min

Detector **:** UV detection at 275 nm

49. **Chi J, *et al.*, (2003)** [68] have developed a sensitive and rapid liquid chromatography tandem mass spectrometry method to measure the levels of the HIV-1 non-nucleoside reverse transcriptase inhibitor nevirapine in human plasma. LC-MS-MS in positive mode used pairs of ions at m/z of 267/226 for nevirapine and 628/421 for the IS respectively.

50. **Droste J A, *et al.*, (2003)** [69] have described a reversed phase high performance liquid chromatography method for the simultaneous quantitative determination of the HIV protease inhibitors and the non-nucleoside reverse transcriptase inhibitor nevirapine in human plasma.

    Column **:** OmniSpher C_{18} column

    Detector **:** UV detection at 215 nm and 280 nm

51. **Jingduan Chi, *et al.*, (2003)** [70] have developed a sensitive method to measure the levels of the NNRTI nevirapine in human plasma by liquid chromatography tandem mass spectrometry.

    Column **:** Zorbax XDB C_8 reverse phase column

    Mobile Phase **:** Buffer pH 4.1 and acetonitrile [gradient flow]

    Flow Rate **:** 400 µL/min

    Mass analyser **:** Triple quadrupole mass spectrometer

52. **Kappelhoff B S, *et al.*, (2003)** [71] have described a simple and rapid high performance liquid chromatographic method for the simultaneous quantification of efavirenz and nevirapine in human plasma suitable for therapeutic drug monitoring. The drugs were separated from endogenous compounds by isocratic reverse phase high performance liquid chromatography with ultraviolet detection at 275 nm.

53. **Rezk N L, *et al.*, (2003)** [72] have reported an accurate, sensitive and specific reverse phase high performance liquid chromatography assay for the simultaneous quantitative determination of the nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitor nevirapine in human

blood plasma. The new polarity dC C_{18} silica column used in this method provides better resolution and peak shape and detected using four different ultraviolet wavelengths for accurate and specific quantitation of the analytes.

54. **Rentsch K M, (2003)** [73] has developed a sensitive and rapid liquid chromatography tandem mass spectrometry method to measure the levels of the HIV-1 non-nucleoside reverse transcriptase inhibitor nevirapine in human plasma. LC-MS-MS in positive mode used pairs of ions at m/z of 267/226 for NVP and 628/421 for the IS, respectively.

55. **A. Volosov, *et al.*, (2002)** [74] have developed simple rapid method for quantification of antiretrovirals by liquid chromatography tandem mass spectrometry.

Column **:** Supelco LC_{18} DB chromatographic column
Mobile Phase **:** Ammonium acetate : methanol
Flow Rate **:** 1 mL/min
Detector **:** UV detection at 260 nm

56. **Fan B, *et al.*, (2002)** [75] have developed a new high-performance liquid chromatography assay for the simultaneous determination of zidovudine/lamivudine/nevirapine in human plasma.

Column **:** Octylsilane column
Mobile Phase **:** Sodium phosphate buffer : acetonitrile [86:14]
Detector **:** UV detection at 265 nm

57. **Laurito T L, *et al.*, (2002)** [76] have developed and validated a rapid, sensitive and specific method to quantify nevirapine in human plasma using dibenzepine as the internal standard. C_{18} analytical column was used with UV detection of 268 nm. This method was employed in a bioequivalence study of two nevirapine tablet formulations.

58. **Langmann P, *et al.*, (2002)** [77] have developed a sensitive and rapid gas chromatographic method to determine the levels of the HIV-1 non-nucleoside reverse transcriptase inhibitor nevirapine in human plasma. CP-Sil 5CB

capillary column with a nitrogen phosphorous detector, Helium 5.0 was used as carrier gas.

59. **Marchei E*, et al.*, (2002)** [78] have developed a simple method for the simultaneous determination of zidovudine and nevirapine in human plasma by reverse phase liquid chromatography.

    Column **:** Zorbax SB C_{18} column

    Mobile Phase **:** Potassium dihydrogen phosphate pH 6.5 : acetonitrile [83 : 17]

    Detector **:** UV detection at 265 nm

60. **Marzolini C*, et al.*, (2002)** [79] have adapted the HPLC method previously described for the simultaneous assay of amprenavir, ritonavir, indinavir, saquinavir, nelfinavir and efavirenz after solid-phase extraction is proposed here for the separate analysis of the newer PI lopinavir and the NNRTI nevirapine.

    Column **:** Nucleosil C_{18} AB

    Mobile Phase **:** Gradient elution acetonitrile: phosphate buffer pH 5.07

    Detector **:** UV detection at 201 and 282 nm

61. **Naser L Rezk, *et al.*, (2002)** [80] have described a reverse phase high performance liquid chromatography assay for the simultaneous quantitative determination of three HIV NNRTI nevirapine, delavirdine, and efavirenz in human blood plasma.

Column **:** Eclipse XDB C_8 column

Mobile Phase **:** Sodium phosphate buffer (pH 4.8) and acetonitrile [Gradient flow]

Flow Rate **:** 1.5 mL/min

Detector **:** UV detection at 224 nm

62. **Poirier J M*, et al.*, (2002)** [81] have developed a sensitive and selective liquid chromatographic assay for the determination of six protease inhibitors active metabolite of nelfinavir and the non-nucleoside reverse transcriptase inhibitor efavirenz. Narrow bore C_{18} reverse phase column was used with gradient

elution. Double ultraviolet detection at 265 nm (amprenavir) and at 210 nm (for all other assayed drugs and internal standard) was used.

63. **Rezk N L, *et al.*, (2002)** [82] have described a simple reverse phase high performance liquid chromatography assay for the simultaneous quantitative determination of three HIV non-nucleoside reverse transcriptase inhibitors (nevirapine, delavirdine and efavirenz) in human blood plasma. Symmetry C_{18} column has been used with UV detection at 270 nm

64. **Titier K, *et al.*, (2002)** [83] have developed a selective and sensitive high performance liquid chromatographic method for the determination of the human immunodeficiency virus protease inhibitors and the non-nucleoside reverse transcriptase inhibitors (nevirapine and efavirenz).

    Column **:** Stability RP C_{18} column

    Mobile Phase **:** Gradient of acetonitrile and phosphate buffer pH 5.65

    Detector **:** UV detection at 240 nm

65. **Tribut O, *et al.*, (2002)** [84] have described a rapid (less than 30 min), sensitive, and specific liquid chromatography method for simultaneous assay of nine anti-retroviral drugs in human plasma. This technique allowed therapeutic drug monitoring of six approved protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir) and two approved non-nucleoside reverse transcriptase inhibitors (efavirenz and nevirapine).

    Column **:** X-TERRA column

    Mobile Phase **:** Water and acetonitrile

    Detector **:** Detection with a diode array detector

66. **Dailly E, *et al.*, (2001)** [85] have reported a single HPLC assay for therapeutic drug monitoring of 5 HIV protease inhibitors (indinavir, amprenavir, saquinavir, ritonavir and nelfinavir) and a non-nucleoside reverse transcriptase inhibitor (nevirapine) in human plasma.

    Column **:** Symmetry C_{18} column

Mobile Phase **:** Gradient elution of solvent A acetonitrile and 0.025 M tetramethyl ammonium perchlorate [55:45] and solvent B methanol

Detector **:** UV detection at 320 nm, 259 nm, 254 nm and 239 nm

67. **Lopez R M, *et al.*, (2001)** [86] have described a fast, simple isocratic reverse phase high performance liquid chromatography method with a 30 mm long column for assaying nevirapine in human serum.

Column **:** C_{18} column

Mobile Phase **:** Phosphate buffer (pH 5) : acetonitrile [82:18]

Flow Rate **:** 1.0 mL/min.

Detector **:** UV detection was performed at 240 nm

68. **Villani P, *et al.*, (2001)** [87] have developed an analytical technique using liquid chromatography (LC) coupled with electrospray mass spectrometry for the simultaneous determination of five protease inhibitors : saquinavir, indinavir, ritonavir, nelfinavir and amprenavir; and three non-nucleoside reverse transcriptase inhibitors : nevirapine, delavirdine and efavirenz, in human plasma.

Column **:** C_{18} reverse phase

Mobile Phase **:** Linear gradient with water and acetonitrile

Detector **:** Mass spectrometry detection

69. **Aymard G, *et al.*, (2000)** [88] have described a new high performance liquid chromatography with UV detection assay for the simultaneous determination of protease inhibitors, nucleoside and non-nucleoside reverse transcriptase inhibitors using a plasma samples.

Column **:** C_{18} symmetry column

Mobile Phase **:** Sodium phosphate buffer : acetonitrile [50:50]

Detector **:** UV detection at 241, 254 and 261 nm

70. **Hollanders R M, *et al.*, (2000)** [89] have reported a sensitive and rapid high performance liquid chromatography method to measure the levels of the nevirapine in human plasma.

Column **:** Hypersil ODS column

Mobile Phase **:** Acetonitrile and phosphate buffer pH 4.5 [30 : 70]

Flow Rate **:** 1.0 mL/min

Detector **:** UV detection at 280 nm

71. **Pav J W, *et al.*, (1999)** [90] have optimized and validated a reverse phase HPLC method for the determination of nevirapine in human plasma, serum, milk and cerebrospinal fluid.

    Column **:** Supelco LC_8 analytical column

    Mobile Phase **:** Phosphate buffer pH 6.0 : methanol : acetonitrile [63 : 21.5 : 15.5]

    Flow Rate **:** 1.0 mL/min

    Detector **:** UV detection at 280 nm

72. **Van Heeswijk R P, *et al.*, (1998)** [91] have described a simple and rapid high performance liquid chromatographic method for the quantification of nevirapine in human plasma. The analyte was separated from endogenous compounds by isocratic reverse phase, ion pair and high performance liquid chromatography with ultraviolet detection at 282 nm.

4. AIM AND PLAN OF WORK

DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR THE ASSAY OF NEVIRAPINE AND ITS RELATED SUBSTANCES IN TABLET DOSAGE FORM

From the literature survey, it was found that there are some analytical methods reported for nevirapine assay and fewer methods for the determination of impurities by RP-HPLC in pharmaceutical dosage forms. Nevirapine is increasingly finding use in treatment of anti-HIV as well as in combination therapy. So there is not only need to develop a newer analytical method for the estimation of nevirapine, but also to identify and quantify its process related impurities and its degradants.

The present work is aimed at

- Method development (By reverse phase HPLC).
- Validation of the developed method (By using following parameters).
    a. Accuracy
    b. Precision
    c. Linearity and Range
    d. Limit of detection
    e. Limit of quantification
    f. Specificity
    g. Robustness
    h. Ruggedness
    i. System suitability studies
    j. Solution stability and filter study

5. INSTRUMENTS AND MATERIALS

The following instruments and materials required for analytical method development and validation process of assay and related substances of nevirapine in tablet dosage form.

Instrument used:

- Waters separation module No. 2695, with PDA detector 2998
- EMPOWER ver.02 software

Reagents used:

1. Monobasic ammonium phosphate : Merck - AR grade
2. Acetonitrile : Merck - HPLC grade
3. Water : Millipore - Milli-Q grade
4. Sodium hydroxide : Merck - AR grade
5. Ethanol : Fischer scientific - AR grade

Standards and samples

Table No: 4 List of standards and sample

Standard / Sample / Impurity Name	**Source**	**Potency / Purity** (%)
Nevirapine USP RS	USP	99.7
Nevirapine Related Compound B	USP	100.0
Nevirapine Related Compound A	USP	100.0
Nevirapine Impurity C	LGC	99.4
Nevirapine USP	Zhejiang Huahai Pharmaceutical	NA
Nevirapine Tablets USP 200 mg	Edict pharma	NA
Nevirapine Tablets USP 200 mg placebo	Edict pharma	NA
Nevirapine for peak identification CRS	Edict Pharma	NA

SUMMARY AND CONCLUSION

Nevirapine is an anti-HIV of the non-nucleoside reverse transcriptase inhibitor (NNRTI). It is more potent than zidovudine on HIV-1, but do not inhibit HIV-2. Viral resistance to these drugs develops by point mutation and cross resistance is common.

Literature reports some UV-visible spectrophotometric, RP-HPLC methods for the quantification of the nevirapine in bulk drugs and formulations. But only few methods are available for determination of related substances and degradants. The chromatographic conditions were optimised for assay as well as related substances and the parameters are,

Mobile phase	: *Monobasic ammonium phosphate buffer pH 5.0: ACN [80 : 20]*
Column	: *Discovery Amide $C_{16,}$ 150 × 4.6, 5 µm*
Flow rate	: *1 mL/ min*
Column temperature	: *35 ºC*
Sample temperature	: *25 ºC*
Injection volume	: *25 µL*
Run time	: *25 mins*
Retention time	: *7.0 mins*
Wavelength	: *220 nm*

The linear response was obtained with acceptable correlation co-efficient. No significant interference seen in specificity study. The method was fully validated for both assay of nevirapine and related substances with the precision, specificity/forced degradation study, accuracy, linearity, ruggedness, robustness, solution stability and filter study.

The values obtained for nevirapine, related compound A, B and impurity C was within the acceptance criteria. Hence the findings indicate that the developed method is precise, specific, accurate, linear, robust and rugged. This method can be used for the routine analysis of nevirapine and its related substances in tablet dosage form.